\_\_\_\_\_\_

Sequence Listing could not be accepted due to errors.

See attached Validation Report.

If you need help call the Patent Electronic Business Center at (866)

217-9197 (toll free).

Reviewer: Anne Corrigan

Timestamp: [year=2008; month=8; day=14; hr=10; min=53; sec=45; ms=828; ]

\_\_\_\_\_

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Reviewer Comments:

<210> 1

<211> 654

<212> DNA

<213> Escherichia coli K12

Please adjust the above <213> response to "Escherichia coli" only, and move the explanatory information to the <220>-<223> section. Per 1.823 of the Sequence Rules, the only valid <213> responses are: the Genus species of the organism, "Artificial Sequence," or "Unknown."

"Artificial Sequence" and "Unknown" require explanation in the <220>- <223> section: please give the source of the genetic material. The above type of response appears in subsequent sequences, too: please adjust them.

<210> 4

<211> 256

<212> PRT

<213> Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

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<221> misc_feature
<222> (250)..(267)
<223> Cys tags
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<223> His tags
<220>
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<223> primer
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1
                                    10
                                                        15
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                                25
                                                    30
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Gly Ser Cys Cys Cys Cys Cys Cys Asp Ile Ile Ser Val Ala Leu Lys

45

40

35

Arg His Ser Thr Lys Ala Phe Asp Ala Ser Lys Lys Leu Thr Pro Glu
50 60

Gln Ala Glu Gln Ile Lys Thr Leu Leu Gln Tyr Ser Pro Ser Ser Thr

70

75

Asn Ser Gln Pro Trp His Phe Ile Val Ala Ser Thr Glu Glu Gly Lys 85 90 95

Ala Arg Val Ala Lys Ser Ala Ala Gly Asn Tyr Val Phe Asn Glu Arg 100 110

Lys Met Leu Asp Ala Ser His Val Val Val Phe Cys Ala Lys Thr Ala 115 120 125

Met Asp Asp Val Trp Leu Lys Leu Val Val Asp Gln Glu Asp Ala Asp 130 140

Gly Arg Phe Ala Thr Pro Glu Ala Lys Ala Ala Asn Asp Lys Gly Arg 145 150 150

Lys Phe Phe Ala Asp Met His Arg Lys Asp Leu His Asp Asp Ala Glu
165 170 175

Trp Met Ala Lys Gln Val Tyr Leu Asn Val Gly Asn Phe Leu Leu Gly 180

Val Ala Ala Leu Gly Leu Asp Ala Val Pro Ile Glu Gly Phe Asp Ala 195 200 205 Ala Ile Leu Asp Ala Glu Phe Gly Leu Lys Glu Lys Gly Tyr Thr Ser 210 220

Leu Val Val Pro Val Gly His His Ser Val Glu Asp Phe Asn Ala 225 230 230 235

Thr Leu Pro Lys Ser Arg Leu Pro Gln Asn Ile Thr Leu Thr Glu Val 245 250 255

Please remove the <220>-<223> sections (as shown below) because Sequence 4 only has 256 amino acids:

<220>

<221> misc\_feature

<222> (250)..(267)

<223> Cys tags

<220>

<221> misc\_feature

<222> (268)..(285)

<223> primer

<220>

<221> misc\_feature

<222> (996)..(1010)

<223> primer

Same type of error in Sequence 6.

(end of Sequence 9)
<400> 9
ggatcctgtt gctgttgctg ttgcgatatc atttctgtcg cc

42

1/11

Please remove the above "1/11" which appears at the end of the submitted file.

\*\*\*\*\*\*\*\*\*\*\*\*

## Validated By CRFValidator v 1.0.3

Application No: 10582557 Version No: 1.0

Input Set:

Output Set:

**Started:** 2008-08-14 09:58:35.104

Finished: 2008-08-14 09:58:36.041

**Elapsed:** 0 hr(s) 0 min(s) 0 sec(s) 937 ms

Total Warnings: 7

Total Errors: 1

No. of SeqIDs Defined: 9

Actual SeqID Count: 9

F	Error code	Error Description
$\nabla$	v 402	Undefined organism found in <213> in SEQ ID (1)
$\nabla$	v 402	Undefined organism found in <213> in SEQ ID (2)
V	V 402	Undefined organism found in <213> in SEQ ID (3)
V	V 402	Undefined organism found in <213> in SEQ ID (4)
V	V 402	Undefined organism found in <213> in SEQ ID (5)
E	201	Mandatory field data missing in <223> in SEQ ID (5)
V	V 402	Undefined organism found in <213> in SEQ ID (6)
V	v 213	Artificial or Unknown found in <213> in SEQ ID (9)

## SEQUENCE LISTING

## <110> University of Wales, Bangor

Trwyn Ltd

<120> Improvements In and Relating to Biosensors

<130> BA/SLH/Y1861

<140> 10582557

<141> 2008-08-14

<160> 9

<170> PatentIn version 3.1

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<213> Escherichia coli K12

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<211> 826

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<213> Pseudomanas putida JLR11

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<222> (88)..(858)

<223> Coding sequence for nfnB gene

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                                                                   114
                             Met Gly Ser Ser His His His His
                             1
                                             5
cac agc agc ctg gtg ccg cgc ggc agc cat atg gct agc atg act
                                                                   162
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
                                       20
                                                           25
10
                   15
ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc gat
                                                                    210
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Asp
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35

40

30

			-	_		_	_	cat His 50						-	_	258
								gcc Ala								306
		_			_			tcc Ser		_					_	354
								cgt Arg								402
						_		atg Met		<u></u>				_		450
		_	_				_	gac Asp 130	_	_		_	_		_	498
								cgc Arg								546
								ttc Phe								594
								atg Met								642
								gcg Ala								690
		_			_	_	_	atc Ile 210								738
						_	_	gtg Val				_				786
								ctg Leu								834
					gaa Glu 255	_	taa	ttct	ctct	tg d	ccggc	gcato	ct go	cccg	gctat	888
++~	at at a	726	a + + ~ +	- aat a	~	-+~~	at a a c		· ~+ + +		0226	7a++ 4	act (	72± 61	at aggg	9/19

ttcctctcag attctcctga tttgcataac cctgtttcag caagcttcgt catcataggc

948

```
tgctgttgaa gcttgcggcc gcactcgagc accaccacca ccaccactga gatccggctg
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Gly	Ser	Cys 35	Cys	Cys	Cys	Cys	Cys 40	Asp	Ile	Ile	Ser	Val 45	Ala	Leu	Lys
Arg	His 50	Ser	Thr	Lys	Ala	Phe 55	Asp	Ala	Ser	Lys	Lys 60	Leu	Thr	Pro	Glu
Gln 65	Ala	Glu	Gln	Ile	Lys 70	Thr	Leu	Leu	Gln	Tyr 75	Ser	Pro	Ser	Ser	Thr 80
Asn	Ser	Gln	Pro	Trp 85	His	Phe	Ile	Val	Ala 90	Ser	Thr	Glu	Glu	Gly 95	Lys
Ala	Arg	Val	Ala 100	Lys	Ser	Ala	Ala	Gly 105	Asn	Tyr	Val	Phe	Asn 110	Glu	Arg
Lys	Met	Leu 115	Asp	Ala	Ser	His	Val 120	Val	Val	Phe	Cys	Ala 125	Lys	Thr	Ala
Met	Asp 130	Asp	Val	Trp	Leu	Lys 135	Leu	Val	Val	Asp	Gln 140	Glu	Asp	Ala	Asp
Gly 145	Arg	Phe	Ala	Thr	Pro 150	Glu	Ala	Lys	Ala	Ala 155	Asn	Asp	Lys	Gly	Arg 160
Lys	Phe	Phe	Ala	Asp 165	Met	His	Arg	Lys	Asp 170	Leu	His	Asp	Asp	Ala 175	Glu
Trp	Met	Ala	Lys 180	Gln	Val	Tyr	Leu	Asn 185	Val	Gly	Asn	Phe	Leu 190	Leu	Gly
Val	Ala	Ala 195	Leu	Gly	Leu	Asp	Ala 200	Val	Pro	Ile	Glu	Gly 205	Phe	Asp	Ala
Ala	Ile 210	Leu	Asp	Ala	Glu	Phe 215	Gly	Leu	Lys	Glu	Lys 220	Gly	Tyr	Thr	Ser
Leu 225	Val	Val	Val	Pro	Val 230	Gly	His	His	Ser	Val 235	Glu	Asp	Phe	Asn	Ala 240
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1

Met Gly Ser Ser His His His His

cac	agc	agc	ggc	ctg	gtg	ccg	cgc	ggc	agc	cat	atg	gct	agc	atg	act	162
His	Ser	Ser	Gly	Leu	Val	Pro	Arg	Gly	Ser	His	Met	Ala	Ser	Met	Thr	
10					15					20					25	
ggt	gga	cag	caa	atg	ggt	cgc	gga	tcc	tgt	tgc	tgt	tgc	tgt	tgc	agc	210
Gly	Gly	Gln	Gln	Met	Gly	Arg	Gly	Ser	Cys	Cys	Cys	Cys	Cys	Cys	Ser	
				30					35					40		
ctt	caa	gac	gaa	gca	ctc	aaa	gcc	tgg	caa	gcc	cgt	tat	ggc	gag	cca	258
Leu	Gln	Asp	Glu	Ala	Leu	Lys	Ala	Trp	Gln	Ala	Arg	Tyr	Gly	Glu	Pro	
			45					50					55			
gct	aac	tta	cct	gct	gcc	gac	acc	gtg	atc	gcg	cag	atg	ttg	cag	cat	306
Ala	Asn	Leu	Pro	Ala	Ala	Asp	Thr	Val	Ile	Ala	Gln	Met	Leu	Gln	His	
		60					65					70				
cga	tca	gta	cgt	gcc	tac	agc	gat	ctt	cct	gtg	gat	gag	cag	atg	ctg	354
Arg	Ser	Val	Arg	Ala	Tyr	Ser	Asp	Leu	Pro	Val	Asp	Glu	Gln	Met	Leu	
_	75		_		_	80	_				85					
aqc	tgg	aca	atc	aca	aca	qcc	caq	tca	qcc	tcq	act	tcc	tcq	aac	ctq	402
	Trp															
90	_				95					100					105	
caa	gct	taa	agc	ata	ctc	acc	ata	caa	gat	cac	gag	cat	ctc	aca	agg	450
	Ala															
0		L	001	110				9	115	9	014	9		120		
									110					120		
ctt	gcc	cga	cta	tcc	aat	aac	cad	cac	cat	atc	gag	cad	gca	cca	cta	498
	Ala															150
шса	1114	2319	125	DCI	Ory	72011	OIII	130	111.5	var	Olu	0111	135		шец	
			120					100					199			
t t c	ctg	ata	t aa	ct c	ata	aac	t aa	tca	cac	cta	cac	caa	cta	acc	ara	546
	Leu															340
rne	цец	140				Asp			AIG	ьeu	ALG	150	пеп	лта	AIG	
		140					140					130				
200	ctt	cac	ac a	cca	act	aca	aat	atc	a a c	tat	tta	a a a	a.c.c	tac	a.c.c	594
	Leu															J J 4
1111	155	GIII	лта	FIO	T 111	160	Сту	116	дър	тут	165	GIU	Ser	тут	1111	
	100					100					100					
at a	ggt	~++	at a	~a±	~~~	aat	at a	aaa	aat	C 2 C	220	aaa	~~~	at a	aat	642
_		_	_	_	_	_	_	_	_	_		_	_		_	042
	Gly	vaı	vaı	Asp		Ата	ьeu	АІА	АІА		ASII	Ата	Ala	ьeu		
170					175					180					185	
++~		~~~	~ ~ ~	or or a	~+ ~	or or a	a + a	~++	+ - ~	a + a	or or or	or or a	a + ar	~~~	228	600
	gag															690
rne	Glu	AId	GTIJ	Ī	ьeu	σтλ	тте	val	_	тте	σтλ	σтλ	met		ASII	
				190					195					200		
	<u> </u>			<u>.</u> L	<b>.</b>		<b></b> -	الساليم		ـــــــــــــــــــــــــــــــــــــ				المنجيج	++-	720
<b>_</b> =	cca	gaa	gcg			gag				_			_			738
		~ ¬	-x -	ъл :	$\sim$		1 - 1 1 1	ьеu	GLV	ьeu	Рro	Asn	ASD	I $n$ $r$	₽n⊖	
	Pro	Glu		Met	Ser	GLU	Olu						_	± <b>11.</b>		
		Glu	Ala 205	Met	Ser	GIU	Olu	210					215	1111	1110	
His	Pro		205					210	_				215			706
His gct	Pro	ttt	205 ggc	atg	tgc	gtc	ggt	210 cat	ccc	gat	ccg	gca	215 cag	ccc	gcc	786
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235 240 245	834
tat gag gcc acc gag gca gag gcg gtt tca gtt gct gcc tat gac cga Tyr Glu Ala Thr Glu Ala Glu Ala Val Ser Val Ala Ala Tyr Asp Arg 250 - 255 - 260 - 265	882
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tcc agc cag gcc gtg gaa cgt gta aaa gga gcg gat tca ctg agc gga Ser Ser Gln Ala Val Glu Arg Val Lys Gly Ala Asp Ser Leu Ser Gly 285 290 295	978
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atacctggca actttgcttg agctccgtcg acaagcttgc ggccgcactc gagcaccacc	1139
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                                                    30
                                25
Gly Ser Cys Cys Cys Cys Cys Ser Leu Gln Asp Glu Ala Leu Lys
        35
                            40
                                                45
Ala Trp Gln Ala Arg Tyr Gly Glu Pro Ala Asn Leu Pro Ala Ala Asp
    50
                        55
                                            60
Thr Val Ile Ala Gln Met Leu Gln His Arg Ser Val Arg Ala Tyr Ser
65
                    70
                                                            80
                                        75
Asp Leu Pro Val Asp Glu Gln Met Leu Ser Trp Ala Ile Ala Ala
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                                    90
                                                        95
Gln Ser Ala Ser Thr Ser Ser Asn Leu Gln Ala Trp Ser Val Leu Ala
                                105
            100
                                                    110
Val Arg Asp Arg Glu Arg Leu Ala Arg Leu Ala Arg Leu Ser Gly Asn
                            120
        115
                                                125
Gln Arg His Val Glu Gln Ala Pro Leu Phe Leu Val Trp Leu Val Asp
    130
                        135
                                            140
Trp Ser Arg Leu Arg Leu Ala Arg Thr Leu Gln Ala Pro Thr Ala
                    150
                                        155
                                                            160
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Gly Ile Asp Tyr Leu Glu Ser Tyr Thr Val Gly Val Val Asp Ala Ala
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                                    170
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Leu Ala Ala Gln Asn Ala Ala Leu Ala Phe Glu Ala Gln Gly Leu Gly
            180
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Ile Val Tyr Ile Gly Gly Met Arg Asn His Pro Glu Ala Met Ser Glu

195 200 205

Glu Leu Gly Leu Pro Asn Asp Thr Phe Ala Val Phe Gly Met Cys Val 210 215 220

Gly His Pro Asp Pro Ala Gln Pro Ala Glu Ile Lys Pro Arg Leu Ala 225 230 230 235 235 240

Gln Ser Val Val Leu His Arg Glu Arg Tyr Glu Ala Thr Glu Ala Glu 245 250 255

Ala Val Ser Val Ala Ala Tyr Asp Arg Arg Met Ser Asp Phe Gln His 260 265 270

Arg Gln Gln Arg Glu Asn Arg Ser Trp Ser Ser Gln Ala Val Glu Arg 275 280 285

Val Lys Gly Ala Asp Ser Leu Ser Gly Arg His Arg Leu Arg Asp Ala